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HYPERANDROGENAEMIA AND FEMALE FERTILITY

REVIEW ARTICLE

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Hyperandrogenaemia and Female Fertility

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Thesis Application to the Degree of Master

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RESUMO

OBJETIVO: Estudar o impacto da hiperandrogenemia na fertilidade da mulher e os mecanismos específicos envolvidos, com especial ênfase no seu papel no ovário.

DESENHO: Foi realizada uma extensa pesquisa na base de dados Pubmed *online* com os termos MeSh relacionados com o tema. A literatura referente ao impacto da hiperandrogenemia na fertilidade feminina foi revista. O nosso objetivo foi focado apenas em seres humanos, especificamente em mulheres, de forma que, estudos em animais e em homens foram excluídos, à exceção de transsexuais expostos a androgénios exógenos, pertencentes à nascença ao sexo feminino. Foram também excluídas mulheres com síndrome do ovário poliquístico, devido ao número de estudos já realizados em grande escala e para percecionar melhor o papel isolado da hiperandrogenemia.

RESULTADOS: A hiperandrogenemia compromete o potencial reprodutivo das mulheres, apesar de haver disparidade entre estudos em células e tecido ovárico em seres humanos. Além disso, a hiperandrogenemia está associada a outros distúrbios metabólicos como a obesidade, a diabetes *mellitus* tipo 2 e dislipidemia.

CONCLUSÃO: A hiperandrogenemia está intrínseca e claramente envolvida nos *outcomes* de (in)fertilidade. Contudo, trata-se de um tema pouco explorado e tivemos dificuldade em encontrar estudos prévios em mulheres que explorassem este tema. Os poucos estudos apresentados nesta revisão são limitados no alcance de mecanismos específicos que poderiam explicar o papel da hiperandrogenemia em mulheres inférteis ou subférteis. São necessários mais estudos em seres humanos sobre questões de fertilidade relacionada com a hiperandrogenemia.

PALAVRAS-CHAVE: hiperandrogenemia; androgénio; ovário; folículos antrais; fertilidade; mulher

ABSTRACT

AIM: To study the impact of hyperandrogenaemia (HA) in women fertility and its specific mechanisms involved, with special emphasis to their role in the ovary.

DESIGN: An extensive search was performed on the online database Pubmed with MeSh terms related to the theme. We reviewed literature regarding the impact of HA in female fertility. Our goal was focused only in humans, specifically women, so animal and men studies were excluded, in exception to female-to-male transsexuals (TSX). We also excluded women with polycystic ovary syndrome (PCOS) due to the large-scale number of studies already been made and to better perceive the HA role alone.

RESULTS: HA impairs women reproductive potential, even though there are disparity between studies in human ovarian cells and tissue. Also, HA is associated with other metabolic diseases, such as obesity, type 2 diabetes *mellitus* and dyslipidaemia.

CONCLUSION: HA is clearly and intrinsically involved in (in)fertility outcomes. However, it is a very unexplored subject and we found it difficult to encounter previous studies in women exploring this theme. The few presented in this review are scarce in achieving the specific mechanisms that could explain HA role in infertile or subfertil women. More human studies are needed about fertility issues related to HA.

KEYWORDS: hyperandrogenaemia; androgen; ovary; antral follicle count; female; fertility

DEDICATION

Aos que, incondicionalmente, amo «por dois».

Pode ouvir-se ainda o seu
bater contra o peito.
Há tantos, tantos anos exposto
à violência da luz do meio
dia. Quase amargo, quase
doce. Só a paixão o rouba
à morte, o impede de ser
panela esburacada
onde o vento assobia.
Ou pior: coisa viscosa, mole,
inerme. Coração,
matéria nobre.

M*atéria nobre | Eugénio de Andrade*

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Aos meus pais, à Clara e ao Bruno, por todo o apoio e carinho durante o percurso académico. Estendo a todos os meus familiares, são dádivas na minha vida. Ao Amadeu, pela ajuda técnica e força constantes. À minha tia Ana e ao meu tio António, por todo o carinho desde a infância.

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À Mira que, mesmo travando uma luta difícil, ocupou-se da minha também, presenteando-me em constância com apoio ímpar.

Para ser grande, sê inteiro: nada
Teu exagera ou exclui.
Sê todo em cada coisa. Põe quanto és
No mínimo que fazes.
Assim em cada lago a lua toda
Brilha, porque alta vive.

Ricardo Reis

ABBREVIATIONS

ACTH – adrenocorticotropic hormone

ADT – androstenedione

AFC – antral follicle count

AMH – anti-Müllerian hormone

AR – androgen receptor

ARs – androgen receptors

ART – assisted reproductive technology

BMI – body mass index

CHO – total cholesterol

DHEA – dehydroepiandrosterone

DHEA-S – dehydroepiandrosterone sulfate

DHT – dihydrotestosterone

FAI – free androgen index

FF – follicular fluid

FPI – fasting plasma insulin

FSH – follicle stimulating hormone

FSH-R – follicle stimulating hormone receptor

GCs – granulosa cells

GDF-9 – growth differentiation factor 9

HA – hyperandrogenaemia

HDL – high-density lipoprotein

ICSI – intracytoplasmic sperm injection

IGF – insulin growth factor

IGF-1 – insulin growth factor 1

LDL – low-density lipoprotein

LH – luteinizing hormone

LH-R – luteinizing hormone receptor

HDL – high-density lipoprotein

HOMA-B – homeostatic model assessment of steady state beta cell function

HOMA-S – homeostatic model assessment

IR – insulin resistance

IVF – *in vitro* fertilization

MD – menstrual disorder

MI – menstrual irregularity

Ob – obese

PCOS – polycystic ovarian syndrome

PP – precocious pubarche

PP14 – placental protein 14

RM – recurrent miscarriages

SHBG – sex-hormone binding globulin

TG – triglycerides

TGF-β – transforming growth factor beta

TNF-α – tumor necrosis factor alfa

TSX – female-to-male transsexuals

TT – testosterone

TFC – total follicle count

T1DM – type 1 diabetes *mellitus*

T2DM – type 2 diabetes *mellitus*

WC – waist circumference

WHR – waist-to-hip ratio

INDEX

INTRODUCTION

Androgens have always been associated to a key functional role in the maintenance of sexual behaviour and male reproduction (Walker, 2009). Nonetheless, online Mendelian Inheritance in Man lists the expression of these steroid hormones in 27 different tissues, including the ovary.

Androgens exert their physiological actions by interaction with their cognate intracellular receptor. The human androgen receptor (AR) gene is located on chromosome Xq11-12 and spans a ~180Kb DNA, which encodes a 110 kDa protein (Lamont and Tindall, 2010) AR functional axis is involved in many human metabolic processes and therefore it is not surprising that AR malfunction plays a central role in the pathogenesis of many diseases including neurodegenerative diseases (Borgia et al., 2017), cancer (Shi et al., 2017) and developmental disorders (Greeley et al., 2017; Ittiwut et al., 2017).

AR deregulation and association to pathogenesis define AR as a major target in hormonally responsive diseases, such as female hyperandrogenaemia.

The present review will present the influence of androgens with focus on its excess in female reproductive physiological processes and fertility potential. Therefore, in order to carry out this task we selected experimental studies from Pubmed database, searching for MeSh (Medicals Subjects Headings) terms related with androgen and ovary. Contributions to sexual health and psychosocial well-being will also be focused.

HYPERANDROGENAEMIA

Hyperandrogenaemia: an overview

Hyperandrogenaemia (HA), or androgen excess, is one of the most common endocrine disturbances in women. HA functional disorders include polycystic ovary syndrome (PCOS), idiopathic hyperandrogenism, idiopathic hirsutism, non-classic congenital adrenal hyperplasia, androgen secreting tumours and other rare disorders (Ehrmann, 2005), whereas HA clinical features consist in male distribution patterns, such as hirsutism and voice changes, acne, and androgenic alopecia (Cousen and Messenger, 2010). The majority of the patients with HA will develop PCOS. Nonetheless, due to the large amount of scientific information devoted to this condition, we will not pursue this subject further. This endocrinopathy affects particularly women of reproductive age. Regarding clinical signs of hyperandrogenism, hirsutism and acne seem to be equally frequent (12.2% prevalence), whereas alopecia is less common (1.7% prevalence). Concerning functional disorders of androgen excess, PCOS and idiopathic hirsutism are equally frequent (5.4% prevalence) followed by idiopathic HA (3.9% prevalence) (Sanchon et al., 2012). Already with regard in non-classic congenital hyperplasia or androgen secreting tumours and other rare disorders these conditions account for 3% and less than 1% of HA cases (Carmina et al., 2006).

Among the clinical manifestations of HA, hirsutism is the one that has the strongest value in the disease diagnostic of women with androgen excess. Hirsutism is defined as excessive growth of terminal hair in women in a male-like pattern (Kuhn, 1972). Its presence is determined through the modified Ferriman-Gallwey score (Ferriman and Gallwey, 1961; Hatch et al., 1981). Hirsutism results from increased androgen production and/or increased sensitivity of the pilosebaceous unit to androgens (Randall, 1994). Reproductive-aged women have tendency to develop more body hair until menopause, while in postmenopausal women this tendency is observed in facial hair. The other manifestations of HA common in women are acne and androgenic alopecia. These androgenic skin changes may be observed independently of hirsutism though these isolated symptoms are not used as a diagnostic criterion for HA. In these cases, an association with hirsutism or menstrual dysfunction should be considered for HA diagnose. Acne is a chronic inflammatory disease of the pilosebaceous unit in which the androgen-induced increased sebum production plays a crucial role (Khondker and Khan, 2014). The cellular and molecular mechanisms by which these hormones exert their influence on the sebaceous gland are hitherto unknown (Shaw, 2002). The severity

of acne is classified accordingly the American Academy of Dermatology guidelines (Pochi et al., 1991). Androgenic alopecia is an androgen-dependent pattern of hair loss, scored through the Ludwig scale (Paik et al., 2001). While in adult women, acne severity is correlated with increased serum androgen levels (Borgia et al., 2004) and acne's absence is observed in women who lack functional ARs (Imperato-McGinley et al., 1993), women with androgenic alopecia have slightly increased serum androgen levels of but increased levels 5α-reductase and ARs, and decreased levels of cytochrome P450 (Drake et al., 1996; Orme et al., 1999; Rebora, 2004; Cousen and Messenger, 2010).

Causes of hyperandrogenaemia

HA can be caused by genetic defects in steroid hormone expression (Mason and Franks, 1997) and primary abnormalities of the hypothalamic-pituitary unit, the ovary and the adrenal gland (Yen, 1980 and Barbieri et al., 1986). Furthermore, although the specific mechanisms remain controversial, obesity has been also linked to HA (Diamanti-Kandarakis and Bergiele, 2001). The proposed underlying mechanisms point out that hyperinsulinemia and insulin resistance (IR) might be involved (Kissebah et al., 1982), having into account the common features between obesity and IR, that is obesity leads to IR and, in turn, IR leads to obesity, both displaying increased hepatic fat deposition (Nicholas et al., 2016).

Not in an independent manner, once connected with obesity as well, hyperinsulinaemia has been also related with HA in reproductive aged women (Burghen et al., 1980; Kissebah et al., 1982; Taylor et al., 1982; Chang et al., 1983). To explain this phenomenon, it is hypothesized that it is hyperinsulinemia the cause of ovarian hyperandrogenism or that hyperandrogenism causes IR and compensatory hyperinsulinemia, or even an unknown factor causes both hyperinsulinemia and hyperandrogenism (Barbieri et al., 1986). Several studies and clinical reports have clearly shown that link but the exact underlying mechanisms are poorly understood (Barbieri and Ryan, 1983; Poretsky, 1991). There is according data mentioning the point of androgen excess production, mainly by the ovaries, as result of hyperinsulinaemia associated with IR. In fact, as described above, insulin is one of the factors involved (Poretsky and Kalin, 1987). In addition, reciprocal potentiation between hyperinsulinemia and HA is postulated (Moghetti et al., 1996). Indeed, insulin and androgen levels act in the ovary in a synergic way, that is, insulin acts to enhance luteinizing hormone (LH) responsiveness leading to greater steroidogenesis (Arthur et al., 1994) and therefore androgen production (Dickerson et al., 2010). Many women with functional ovarian

hyperandrogenism have hyperinsulinemia (Burghen et al., 1980; Taylor et al., 1982; Chang et al., 1983). In these women, insulin may act alone or synergistically with LH to increase ovarian androgen production (Barbieri et al., 1986).

The implication of this metabolic disturbance in the feminine reproductive system

Androgens are produced in different organs but most of their production takes place in the adrenal glands and in the ovary (Lebbe & Woodruff, 2013). The concentration ranges for testosterone (TT) is 0.5-2.0 nmol/l and its precursor, androstenedione (ADT) is 3.0-7.0 nmol/l. Pathways of androgen production in thecal and granulosa cells are reviewed by Norman (Norman, 2002). Though the exact role of androgens in the female reproductive system remains unclear, it is of knowledge that HA exerts a negative impact (Arlt, 2006).

The *zona reticularis* of the adrenal gland cortex produces the androgens dehydroepiandrosterone (DHEA), an important precursor of sex steroids and ADT, activated by the pituitary cortical androgen-stimulating hormone and insulin growth factor 1 (IGF-1) (Gurnell et al., 2008; Sá and Sousa, 2014). The DHEA is the unic source of sex steroids for all tissues, except the utherus, in postmenopausal women. For this reason, it seems that DHEA might be involved in mechanisms related with the symptoms found after menopause, such as vaginal atrophy, bone loss, fat accumulation, type 2 diabetes *mellitus* (T2DM), skin atrophy, cognition problems and memory loss (Labrie, 2010). The adrenal gland responds to adrenocorticotropic hormone (ACTH) (Gebre-Medhin et al., 2000).

In the ovary, androgen production occurs in theca cells and in the ovarian stroma. Several substances are thought to participate in the increase of androgen levels, including LH, activin, tumour necrosis factor alpha (TNF-α), Insulin growth factors (IGFs) and interleukins (Norman, 2002). LH release stimulates the ovary to raise the production of 17-α-hydroxyprogesterone, which is overproduced in case of HA. Activin, a dimeric protein of β-subunits that belongs to the transforming growth factor β (TGF-β) family, plays a role in the development and continued existence of healthy oestrogenic follicles, preventing premature luteinisation. In fact, activin allows that the follicle stimulating hormone receptor (FSH-R) starts to be expressed in the granulosa cells (GCs) in the primary follicle. FSH-R are present in all GCs since the secondary follicle stage, where the growth differentiation factor 9 (GDF-9), also a TGF-β secreted by the oocyte, acts

through mitogenic effect, allowing GCs growing. GCs are the only cells to express FSH-R. Another molecule secreted by GCs is KIT-L, involved in the formation of the theca interna (Sá and Sousa, 2014). Inversingly, follistatin leads to the luteinisation process (Findlay, 1994). TNF-α is one of the follicular fluid components, but it remains unclear if alteration of TNF-α levels interfere with oocyte developmental competence. Also, TNF-α functions consist in apoptosis regulation, which leads to follicular atresia (Roby and Terranova, 1988; Adashi et al., 1990; Wang et al., 1992; Van Voorhis et al., 1994; Lee et al., 2000). IGFs play an important role on granulosa cell cytodifferentiation (Garzo and Dorrington, 1984). Glister et al. reported the importance of IGF peptide 3 in keeping androgen production by theca cells and that the inhibitory action of bone morphogenic proteins on androgen production is related to their inhibition role on ILP-3 signalling, which seems to be mediated via down-regulation of the nuclear transcription factor steroidogenic factor-1 (Glister et al., 2013). Also, it has been reported that androgen production is also achieved by hormone stimulation; insulin and IGF-1 are able to stimulation of androgen production (Barbieri et al., 1986). Angiogenesis is very important and depends on the vascular endothelial growth factor, with the urge of blood vessels in preantral theca interna GCs.

Regarding female fertility, the ovary production of androgens plays a central role (Glister et al., 2013). Thus, it is important to be aware of its known physiology and biochemical paths. Folliculogenesis and steroidogenesis is schematically represented in **Figure 1**. Each pathway potentially could be the causal HA, when disturbed.

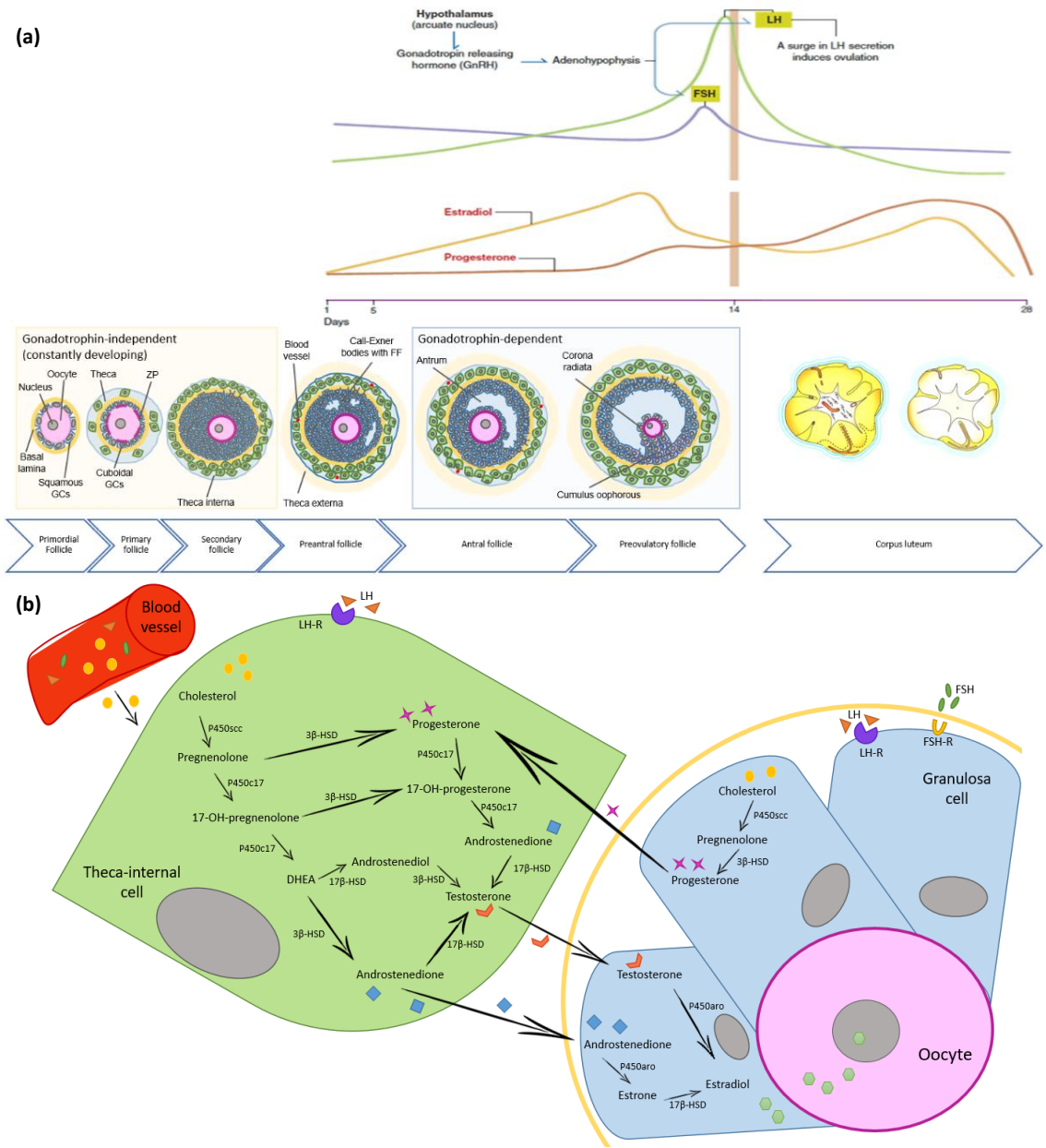

Figure 1. **(a)** Menstrual cycle and Folliculogenesis. **(b)** Steroidogenesis. LH, luteinizing hormone; LH-R, LH receptor; FF, follicular fluid; FSH, follicle stimulating hormone; FSH-R, FSH receptor; GCs, granulosa cells; P450c17, cytochrome P450c17; ZP, zona pellucida; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase. Adapted from Leão et al., 2014; Li et al., 2014; Sá and Sousa, 2014 and Kierszenbaum et al., 2016.

Androgen levels are higher in the mid cycle phase of the menstrual cycle: also, they decrease with age or ovary removal (Norman, 2002). Androgen receptors (ARs) are expressed in all types of cells present in the ovarian follicle, including the oocyte, granulosa and theca cells and the cortical stroma (Sen and Hammes, 2010). Increasing AR concentrations are detected in granulosa cells (GCs) from the primary stage onward, peaking in the antral stage (Sen and Hammes, 2010). Several growth factors and

cytokines act in the ovary in autocrine or paracrine manner (Rice et al., 2007). TT and 5-dyhidrotestosterone (5-DHT) are the only hormones capable to bound to AR. Thus, androgens have an important role in folliculogenesis and its excess might infere with folliculogenesis normal course. LH and FSH pulses, through hypothalamus hypophyses axis, were already been well documented by Rojas et al. (Rojas et al., 2015).

Noteworthy, the exact role of androgens in women remains controversial (Norman, 2002). Contributions to sexual health and well-being have been mentioned (Arlt et al., 1999; Gebre-Medhin et al., 2000; Norman, 2002) and will be discussed below, despite no further clarification of the underlying mechanisms involved was added.

HYPERANDROGENAEMIA AND FEMALE REPRODUCTIVE POTENTIAL

Female HA is well recognized to play detrimental impact on fertility (Lebbe and Woodruff, 2013). However, its underlying mechanisms are poorly understood. Our aim was to understand more clearly the impact of HA in fertility, with emphasis to the specific mechanisms that might be involved. Studying the human ovary and its constituents is truly challenging. Indeed, on one hand, besides ethical issues in human ovarian tissue management, the HA focus has been in large scale applied to PCOS. As mentioned before, this consequence of HA was excluded of our scope of work. On the other hand, to assemble human oocytes studies concerned about the particular mechanisms involved is like to find *«a needle in a haystack»*. The articles selected having this matter into account were very low, in the view of the extensive search, and are listed in **Table 1.** Already in 1990, Apter and Vihko mentioned the necessity of more studies, reporting the association between HA and poorer fertility outcomes: women with HA had lower pregnancy rates and it seemed that HA was connected to previous HA in puberty (Apter and Vihko, 1990).

Table 1: Hyperandrogenaemia consequences on female fertility potential.

Reference	Type of work	Country	Aim	Study group	Relations
Cui et al., 2016_RE	Cross-sectional study	China	Age-specific AMH distribution Association of AMH with androgenic and metabolic profiles at different ages	6763 women, 0-64y, divided in 4 phases: Childhood (0-10y, n=739) Adolescence (11-18y, n=471) Reproductive age (19-50y, n=5500) Advanced age (>51y, n=53)	AMH: ↑ until peak at 18y, ↓ during the reproductive age period; low level above 0 onward; Negatively correlated with TT in childhood; Positively correlated with TT and FAI (adolescence and reproductive phases); Not correlated to BMI, IR or dyslipidaemia.
Hvidman et al., 2016_HR	Prospective cohort study	Denmark	Do infertile patients <40y have a lower ovarian reserve, estimated by AMH and AFC?	382 infertile women 350 without history of infertility, control group	Infertile patients had similar AMH levels and AFC
West et al., 2014_HR	Prospective cohort Finland		Do teen girls with MI have ↑androgen levels and ↑risk of infertility?	16y girls and later 26y, n=2033 (questionnaire) at 16y with MI (n=482) at 16y without MI (n=1252)	At the age of 26y, MI and androgenism signs when 16y have: Hirsutism: ↓ [SHBG] ↑ FAI Acne: ↑[TT] ↓ [SHBG] ↑ FAI No link to ↑ risk of childlessness
Irez et al., 2011_AGO	Clinical trial	Turkey	Association between different of AMH $_{basal}$ levels Oocyte-embryo quality and IVF outcomes	209 infertile women undergoing IVF with ICSI	AMH: Negative correlation with age; Positive correlation with gonadotropin, AFC and the number of oocytes; Ongoing pregnancy rate > in percentiles 25-90%.
Dickerson et al., 2010_HR	Prospective study	UK	Influence of IR and androgen status in non-PCOS women	Infertile women without PCOS, with regular menstrual cycle, undergoing an ART cycle, n=49	Positive correlation with FAI, TT and BMI. Positive correlation of TFC and FAI. Negative correlation of HOMA with SHBG. No correlation between HOMA or FPI and TFC. No correlations between number of oocytes retrieved, fertilization or cleavage rates and FPI, HOMA and androgen levels.
Eilso Nielsen et al., 2010_MHR	Cohort study	Denmark Japan	Determine expression of AMH-r2 and correlate to expression of CYP19, FSH-R and LH-R and to the normal profile	24 women, 64 follicles, 15-37y	In FF, AMH levels (ligand) had a negative correlation with mRNA expression of the aromatase (CYP 19); [AMH] had no correlation with androgens. In GC, AMH-r2 has a positive correlation with FSH-R expression and no correlation with androgens. Negative correlation with progesterone.
Nardo et al., 2009_HR	Cohort study	UK	Relationship between IR, androgens and AMH AMH contributes to altered folliculogenesis?	232 women, 22-41y, IVF work-up prior to 1rst IVF treatment, n=232 non-PCOS women, n=183	AMH levels: ↑ with AFC, LH, insulin and TT levels, FAI and HOMA-IR; AMH levels ↓ with HOMA-B and SHBG levels
Ibáñez et al., 2002_TJCEM	Prospective Cohort study	Spain UK Belgium	Novel therapeutic avenues of mechanisms link hyperinsulinemic hyperandrogenism	31 HA and hyperinsulinemic women, 18-22y, 9 months: 10 FT, flutamide monotherapy 8 MT, metformin monotherapy 13 CT, flutamide-metformin combined therapy	CT women: <hirsutism, androgen levels and dyslipidemia than women who received FT <anovulatory cycles
Okon et al., 1998_FS	Retrospective study	USA	[Androgen]$_{plasma}$ levels in RM Correlate to [PP14] $_{uterine}$ and [PP14]$_{plasma}$	43 non-PCOS women with RM, 20-40y 18 women without RM, control group	>[TT] on days LH-7 and LH-4; >[ADT] on day LH-7; >TT SHBG ratios in women with RM on days LH-7, LH+0 and LH+7. Negative correlation between [TT]$_{follicular}$ and [androgen]$_{plasma}$ and PP14$_{uterine}$
Pache et al., 1991_RH	Case control	Netherlands	Androgens action in the ovary	17 women TSX (29 ovaries) 13 regularly cycle women (14 ovaries)	- ovaries enlarged - ↑cystic follicle (2x) - ↑atretic follicle (3.5x) - ↑collagenized and thicker (3x) cortex
Apter and Vihko, 1990_JCES	Longitudinal cohort study (1975→1987)	Finland	Endocrine features and factors related to fertility	200 girls, 7-17y→not accomplished pregnancy women, n=26 *vs*. women who became pregnant, n=18, 20-30y	↓pregnancy rate HA correlated with previous HA in puberty

AFC, antral follicle count; AMH, anti-Müllerian hormone; AMH-r2, anti-Müllerian hormone receptor 2; BMI, body mass index; CYP19, cytochrome-P-19; FAI, free androgen index; FF, follicular fluid; FPI, fasting plasma insulin; FSH-r, follicle stimulating receptor; HDL, high-density lipoprotein; HA, hyperandrogenaemia; HOMA, homeostatic model assessment; ICSI, intracytoplasmic sperm injection; IR, insulin resistance; IVF, *in vitro* fertilization; LDL, low-density lipoprotein; LH-r, luteinizing hormone receptor; MD, menstrual disorder; MI, menstrual irregularity; Ob, obese; PP14, placental protein 14; RM, recurrent miscarriage; SHBG, sex-hormone bind globulin; TFC, total follicle count; TSX, female-to-male transsexuals; TT, testosterone; WC, waist circumference; WHR, waist-hip ratio.

HA link to recurrent miscarriages (RM) was pointed out (Okon et al., 1998; Cocksedge et al., 2008). Okon et al., 1998 went further and hypothesized a mechanism that could explain this association: HA might result in an abnormal secretory endometrium through high androgens concentration with impact on placental protein 14 (PP14) production, a protein involved in embryo implantation, lowering its uterine levels. Also, they obtained higher concentrations of ADT in the follicular phase in women with RM (Okon et al., 1998). Pache et al. had the opportunity to study ovaries from female-to-male transsexuals (TSX), that is, exposed to higher exogenous androgen levels for some period. The result in ovarian TSX tissue was an alteration in follicle development (follicle growth and follicle arrest induction, thicker theca interna), collagenization and thickening of the ovarian cortex and the well-recognized cystic alterations (stromal and theca interna hyperplasia) (Pache et al., 1991). Thus, both exogenous and endogenous androgen excess exposure decreases reproductive outcomes. Recently, Panidis et al. reviewed this theme as a two-sided capacity of androgens dependency on its levels. In normal levels, androgens are essential for follicle development but in hyperandrogenism, it can be compromised (Panidis et al., 2015). In agreement, Prizant et al., also mentioned the importance of a balance in androgen levels to normal reproductive function (Prizant et al., 2014). The negative effects of androgens on follicle development have been reviewed by Gleicher et al., 2011. It seems that androgens negative impact is more pronounced in the earliest stages and two ways to explain it are presented: the genomic, through ARs, and the non-genomic, for example diffusion (Gleicher et al., 2011).

Efforts have exhaustively been made to understand if anti-Müllerian hormone (AMH) can be a fiable marker of the human ovarian reserve, which has a tremendous prognostic value in women who seek to conceive. Decreased ovarian reserve compromises *in vitro* fertilization (IVF) success once it is associated to aneuploidy, embryo defective development, lower implantation success and higher spontaneous miscarriages (Irez et al., 2011; Lebbe and Woodruff, 2013). This can be explained by the correlations between AMH concentrations and the human ovarian reserve. Also, age is related to ovarian reserve. Indeed, the human ovarian reserve decreases along the years until menopause. With the decrease of the ovarian reserve, the oocyte quality also decreases because of women aging. Thus, oocyte morphology and chromosome anomalies such as aneuploidy occur, which leads to higher number of spontaneous abortion, to embryo and fetal compromised development and to decrease in the number of oocyte achieving metaphase II stage. Androgens are also regarded as one of the factors that might affect the ovarian reserve and recently, it has been postulated that androgens exert effect in the regulation of AMH expression and/or levels (Lebbe and

Woodruff, 2013). Thus, its correlation to AMH from adolescent to reproductive stages leads researchers to believe that there is a link between AMH and androgens (Cui et al., 2016). Nardo et al. demonstrated this association in infertile women (Nardo et al., 2009). Eilso Nielson obtained no correlations between AMH and androgens, but probably this can be explained by the fact that the FF was extracted from small antral follicles from ovaries removed surgically (Eilso Nielsen et al., 2010). Irez et al. studied different AMH concentrations in women under IVF and concluded that AMH in extreme concentrations, that is, under 1.4 ng/mL and above 8.06mg/mL were associated to poorer ongoing pregnancies (Irez et al., 2011). It is of importance that AMH concentrations are studied once its helps to predict the oocyte quality and assisted reproductive technology outcomes. For instance, it is known that higher concentrations of AMH mean that more follicles are available to be stimulated to mature, that is, the ovarian reserve is higher. Recently, Hvidman et al. studied women under forty years-old to an attempt to establish a possible association between AMH levels and AFC. AMH levels were not correlated to AFC, despite previously positive correlations between AMH levels and AFC found by Irez et al. and Nardo et al. (Nardo et al., 2009; Irez et al., 2011; Hvidman et al., 2016). Once again, a struggle is patent in finding the pathways implied.

HA might be the responsible or even the consequence of other metabolic disorders. Ibánez et al. studied hyperinsulinaemic and hyperandrogenaemic women receiving combined therapy flutamide-metformin, anti-androgen and insulin sensitizing drugs, respectively. The authors concluded that women who received combined therapy had higher ovulatory cycles than women who received monotherapy. This phenomenon leads to think that HA is related to hyperinsulinaemia, and thus to IR, and that both alter female fertility. The concomitant dyslipidemia seemed to be a consequence of HA in the presence of hyperinsulinaemia (Ibanez et al., 2002). As we noticed, the intricate relations to other metabolic disorders, and its relations are listed in **Table 2**.

Table 2: Hyperandrogenaemia and its relations to other metabolic disturbances.

Reference	Type of work	Country	Aim	Study group	Results
Pinola et al., 2012_HR	Cohort study Questionnaire	Finland	Are self-reported menstrual disorders associated with HA and metabolic disturbances as early as in adolescence?	Teen girls, 15-16 y, n=2448 MD group, with menstrual disorder, n=709 Without MD, n=1739	> androgen levels in MD women Whole population study: Positive correlation between BMI and WHR, FAI, FPI, TG and LDL Negative correlation between BMI and HDL, SHBG and Negative correlation between HOMA-S and age at menarche
Samara-Boustani et al., 2012_EJE	Cross-sectional study	France	The pubertal development, hormonal profiles and prevalence of hirsutism and MD between Ob and T1DM	Ob adolescent girls, n=96 Adolescent girls with T1DM, n=78	Ob girls: < age at pubarche, thelarche and menarche >hirsutism No difference in androgen levels between hirsutism and MD groups > androgen levels in hirsute obese women
Yasmin et al., 2012_EJOGRB	Cross-sectional, observational study	UK	Association between BMI and different androgen parameters	121 Ovulatory women BMI≤30 Kg/m^2, n=95 BMI>30 Kg/m^2, n=37	Positive correlations between BMI and FAI Negative correlations between BMI and SHBG No correlations between BMI and TT ↓↓insulin sensitivity with ↑ BMI
Zhou et al., 2012_CE	Cross-sectional study	China	Normal cut-off value for the FAI in reproductive-age Chinese women	1526 women ,20-45y Control group, n=444, non-HA	HA women: > BMI, acne score, androgen levels, CHOL and TG; > MI, hirsutism, acne, IR or DM, Ob and fatty liver
Wei et al., 2010_BMCMD	Cross-sectional study	Australia	Associations between menstrual pattern, TT, SHBG, FAI and bone mass	Women, n=382 (11%), 26-36y, menstrual pattern (questionnaire) with irregular cycles, n=41 with regular cycle n=341	>FAI and <SHBG in MI women
Wei et al., 2009_O	Cross-sectional study	Australia	Association between BMI, WC, WHR and menstrual cycle pattern with hormonal factors	726 women,26-36y with different body composition measures and menstrual cycle pattern (questionnaire)	BMI, WC and WHR were positively correlated with androgen and insulin levels.
McCartner et al., 2006_JCEM	Cross-sectional study	USA	Is pubertal obesity associated with HA?	Peripubertal girls, n=76, 7-17y Ob group, n=41 Non-obese group, n=35	>androgen levels in obese women Early pubertal girls: BMI correlated positively with androgen and insulin levels
Ibáñez et al., 1999_CE	Cohort study	Spain	Can abnormal IR, Dyslipidemia and HA also be detected in 1^{rst} degree relatives of PP girls?	1^{rst} degree relatives of PP girls, n=60 Women, n=30 Hirsutism, n=10 , 5 with MI Non-hirsutism, n=20, 2 with MI Control group Men, n=30	Hirsute woman with MI relatives >androgen levels in >abnormal glucose tolerance poor lipid profile

ADT, androstenedione; AFC, antral follicle count; AFM, abdominal fat mass; AMH, anti-Müllerian hormone; BMI, body mass index; CHOL, cholesterol; DHEA-S, dehydroepiandosterone sulphate; FAI, free androgen index; FF, follicular fluid; HA, hyperandrogenaemia; HDL, high-density lipoprotein; LDL, low density lipoprotein; HOMA-S, homeostasis model assessment; IR, insulin resistance; LDL, low-density lipoprotein; MD, menstrual disorder; MI, menstrual irregularity; Ob, obese; PP, precocious pubarche; SHBG, sex-hormone bind globulin; TG, triglycerides; TT, testosterone; T1DM, Type 1 diabetes mellitus; WC, waist circumference; WHR, waist-hip ratio.

HA is reported to be related to menstrual irregularity (MI) in several studies (Wei et al., 2009; Wei et al., 2010; Pinola et al., 2012) which is associated to anovulation. However, we need to bear in mind that menses regularity was in some studies achieved by questionnaire, which is a limitative aspect. Recently, West et al. performed a 2033 women prospective cohort to verify if teenage girls with MI at the age of 16 years-old would have HA and impaired reproductive outcomes at the age of 26 years-old. Teen girls with hirsutism and acne at 16 years-old displayed elevated androgen levels later, which suggests the early onset of HA (West et al., 2014).

Of interest, considering the obesity pandemics worldwide and the remarkable increase in T2DM as well, it is alarming to realize that hyperinsulinemia related to obesity might contribute to HA (Anderson et al., 2014). Ibáñez et al. suggested that there might be a genetic basis for this association, based in the higher prevalence of dyslipidemia, IR, and HA in first degree relatives of girls with a history of precocious pubarche (PP) (Ibanez et al., 1999). Furthermore, in a posterior study, Ibáñez and colleagues also studying girls with PP mentioned that abdominal fat mass and truncal fat mass were positive related to fast plasma insulin, androgen and lipid levels (Ibanez et al., 2003). Panidis et al. also had results consistent with these correlations between IR, obesity and HA, although in a small sample of women (Panidis et al., 2015). The influence of IR and in androgen excess was studied by Dickerson et al. They showed that in women without PCOS, IR and HA can have impact in ovary function. Specifically, it is proposed that relatively higher level of insulin stimulates follicle recruitment or development (Dickerson et al., 2010). Noteworthily, Zhou et al. found higher prevalence of IR or T2DM, obesity and fatty liver in a large sample of HA women in the reproductive age, which gives further information pointing to a correlation between HA and metabolic disorders (Zhou et al., 2012).

MacCartner et al. studied obesity in peripubertal girls and its influence in androgen levels. They had even higher androgen levels in early puberty (McCartney et al., 2006). Also, as mentioned by Cockesedge et al., women with higher free androgen index (FAI) have higher body mass index (BMI), which supports the correlation between HA and obesity (Cocksedge et al., 2008). Furthermore, BMI, waist circumference (WC) and waist-to-hip (WHR) ratio were positively correlated with FAI and insulin levels. Fasting plasma glucose association with androgens is consistent with the literature (Wei et al., 2009). This phenomenon was also observed by Samara-Boustani et al., studying an obese group of adolescent girls which showed earlier age of pubarche, thelarche and

menarche and higher prevalence of MI and hirsutism and androgen levels (Samara-Boustani et al., 2012). In addition. a link between BMI and HA seems to deserve attention, but the related mechanisms have not been clearly understood yet (Yasmin et al., 2013).

We suggest that the relations with androgens are not independent and one single piece can interfere in several aspects so the exactly first step that can lead to female infertility is difficult to announce.

IMPACT OF HYPERANDROGENAEMIA ON WOMEN MORBIDITY, QUALITY OF LIFE AND ECONOMICS

HA can negatively impact the quality of life of the affected women, being considered as a major cause of psychologic distress, with self-confidence, self-esteem and psychosexual implications that lead to changes in their lifestyle. It is also known that HA brings pronounced emotional repercussions, such as depression, frustration and anxiety, that lead to social eviction (Sonino et al., 1993; Girman et al., 1999; Thomas, 2004).

Also, as we previously discussed, HA seems to be linked with other metabolic disturbances, such as IR and T2DM, dyslipidaemia and obesity, which constitute high prevalent issues in terms of morbidity and financial costs to the patient and to the national health system. In Portugal, 8495 mothers who gave birth have been studied. About 33% of them were overweight or obese before pregnancy (Alves et al., 2012). This is in agreement with the pandemics of obesity worldwide, with particular relief not only in decreased female fertility concomitantly with its association and probable cause of HA as we mentioned in this review, but also with consequences in embryo development (macrosomia, for instance) and in post-birth life, namely with higher prevalence of metabolic disturbances in the descendance.

Therefore, HA constitutes a socioeconomic problem, as it affects this women's behaviour and interaction with others and conducts to health consultations in several areas (Sonino et al., 1993).

CONCLUSIONS

HA has an effective and important role in female fertility. However, it is hard to study this matter due to the lack of research reporting the specific underlying mechanisms. Those existing, indeed state that the excess in androgens can have a negative impact on miscarriage rate, ovarian reserve, follicle development, implantation success and pregnancy rates. Also, other metabolic disorders, such as obesity, hyperinsulinemia, T2DM, and dyslipidaemia seem to be in intimate relation with HA.

REFERENCES

Adashi, E. Y., Resnick, C. E., Packman, J. N., Hurwitz, A. and Payne, D. W. (1990). Cytokine-mediated regulation of ovarian function: tumor necrosis factor alpha inhibits gonadotropin-supported progesterone accumulation by differentiating and luteinized murine granulosa cells. *Am J Obstet Gynecol, 162*(4), 889-896; discussion 896-889.

Alves, E., Correia, S., Barros, H. and Azevedo, A. (2012). Prevalence of self-reported cardiovascular risk factors in Portuguese women: a survey after delivery. *Int J Public Health, 57*(5), 837-847.

Anderson, A. D., Solorzano, C. M. and McCartney, C. R. (2014). Childhood obesity and its impact on the development of adolescent PCOS. *Semin Reprod Med, 32*(3), 202-213.

Apter, D. and Vihko, R. (1990). Endocrine determinants of fertility: serum androgen concentrations during follow-up of adolescents into the third decade of life. *J Clin Endocrinol Metab, 71*(4), 970-974.

Arlt, W. (2006). Androgen therapy in women. *Eur J Endocrinol, 154*(1), 1-11.

Arlt, W., Callies, F., van Vlijmen, J. C., Koehler, I., Reincke, M., Bidlingmaier, M. et al. (1999). Dehydroepiandrosterone replacement in women with adrenal insufficiency. *N Engl J Med, 341*(14), 1013-1020.

Arthur, I. D., Anthony, F. W., Masson, G. M. and Thomas, E. J. (1994). The influence of ovarian follicular activity on late proliferative phase serum IGFBP-1 in down-regulated assisted conception cycles. *Hum Reprod, 9*(8), 1417-1420.

Barbieri, R. L., Makris, A., Randall, R. W., Daniels, G., Kistner, R. W. and Ryan, K. J. (1986). Insulin stimulates androgen accumulation in incubations of ovarian stroma obtained from women with hyperandrogenism. *J Clin Endocrinol Metab, 62*(5), 904-910.

Barbieri, R. L. and Ryan, K. J. (1983). Hyperandrogenism, insulin resistance, and acanthosis nigricans syndrome: a common endocrinopathy with distinct pathophysiologic features. *Am J Obstet Gynecol, 147*(1), 90-101.

Borgia, D., Malena, A., Spinazzi, M., Desbats, M. A., Salviati, L., Russell, A. P. et al. (2017). Increased mitophagy in the skeletal muscle of spinal and bulbar muscular atrophy patients. *Hum Mol Genet, 26*(6), 1087-1103.

Borgia, F., Cannavo, S., Guarneri, F., Cannavo, S. P., Vaccaro, M. and Guarneri, B. (2004). Correlation between endocrinological parameters and acne severity in adult women. *Acta Derm Venereol, 84*(3), 201-204.

Burghen, G. A., Givens, J. R. and Kitabchi, A. E. (1980). Correlation of hyperandrogenism with hyperinsulinism in polycystic ovarian disease. *J Clin Endocrinol Metab, 50*(1), 113-116.

Carmina, E., Rosato, F., Janni, A., Rizzo, M. and Longo, R. A. (2006). Extensive clinical experience: relative prevalence of different androgen excess disorders in 950 women referred because of clinical hyperandrogenism. *J Clin Endocrinol Metab, 91*(1), 2-6.

Chang, R. J., Nakamura, R. M., Judd, H. L. and Kaplan, S. A. (1983). Insulin resistance in nonobese patients with polycystic ovarian disease. *J Clin Endocrinol Metab, 57*(2), 356-359.

Cocksedge, K. A., Saravelos, S. H., Wang, Q., Tuckerman, E., Laird, S. M. and Li, T. C. (2008). Does free androgen index predict subsequent pregnancy outcome in women with recurrent miscarriage? *Hum Reprod, 23*(4), 797-802.

Cousen, P. and Messenger, A. (2010). Female pattern hair loss in complete androgen insensitivity syndrome. *Br J Dermatol, 162*(5), 1135-1137.

Cui, L., Qin, Y., Gao, X., Lu, J., Geng, L., Ding, L. et al. (2016). Antimullerian hormone: correlation with age and androgenic and metabolic factors in women from birth to postmenopause. *Fertil Steril, 105*(2), 481-485.e481.

Diamanti-Kandarakis, E. and Bergiele, A. (2001). The influence of obesity on hyperandrogenism and infertility in the female. *Obes Rev, 2*(4), 231-238.

Dickerson, E. H., Cho, L. W., Maguiness, S. D., Killick, S. L., Robinson, J. and Atkin, S. L. (2010). Insulin resistance and free androgen index correlate with the outcome of controlled ovarian hyperstimulation in non-PCOS women undergoing IVF. *Hum Reprod, 25*(2), 504-509.

Drake, L. A., Dinehart, S. M., Farmer, E. R., Goltz, R. W., Graham, G. F., Hordinsky, M. K. et al. (1996). Guidelines of care for androgenetic alopecia. American Academy of Dermatology. *J Am Acad Dermatol, 35*(3 Pt 1), 465-469.

Ehrmann, D. A. (2005). Polycystic ovary syndrome. *N Engl J Med, 352*(12), 1223-1236.

Eilso Nielsen, M., Rasmussen, I. A., Fukuda, M., Westergaard, L. G. and Yding Andersen, C. (2010). Concentrations of anti-Mullerian hormone in fluid from small human antral follicles show a negative correlation with CYP19 mRNA expression in the corresponding granulosa cells. *Mol Hum Reprod, 16*(9), 637-643.

Ferriman, D. and Gallwey, J. D. (1961). Clinical assessment of body hair growth in women. *J Clin Endocrinol Metab, 21*, 1440-1447.

Findlay, J. K. (1994). Peripheral and local regulators of folliculogenesis. *Reprod Fertil Dev, 6*(2), 127-139.

Garzo, V. G., & Dorrington, J. H. (1984). Aromatase activity in human granulosa cells during follicular development and the modulation by follicle-stimulating hormone and insulin. *Am J Obstet Gynecol, 148*(5), 657-662.

Gebre-Medhin, G., Husebye, E. S., Mallmin, H., Helstrom, L., Berne, C., Karlsson, F. A. and Kampe, O. (2000). Oral dehydroepiandrosterone (DHEA) replacement therapy in women with Addison's disease. *Clin Endocrinol (Oxf), 52*(6), 775-780.

Girman, C. J., Hartmaier, S., Roberts, J., Bergfeld, W. and Waldstreicher, J. (1999). Patient-perceived importance of negative effects of androgenetic alopecia in women. *J Womens Health Gend Based Med, 8*(8), 1091-1095.

Gleicher, N., Weghofer, A. and Barad, D. H. (2011). The role of androgens in follicle maturation and ovulation induction: friend or foe of infertility treatment? *Reprod Biol Endocrinol, 9*, 116.

Glister, C., Satchell, L., Bathgate, R. A., Wade, J. D., Dai, Y., Ivell, R. et al. (2013). Functional link between bone morphogenetic proteins and insulin-like peptide 3 signaling in modulating ovarian androgen production. *Proc Natl Acad Sci U S A, 110*(15), E1426-1435.

Greeley, S. A., Littlejohn, E., Husain, A. N., Waggoner, D., Gundeti, M., & Rosenfield, R. L. (2017). The Effect of the Testis on the Ovary: Structure-Function Relationships in a Neonate with a Unilateral Ovotestis (Ovotesticular Disorder of Sex Development). *Horm Res Paediatr, 87*(3), 205-212.

Hatch, R., Rosenfield, R. L., Kim, M. H. and Tredway, D. (1981). Hirsutism: implications, etiology, and management. *Am J Obstet Gynecol, 140*(7), 815-830.

Hvidman, H. W., Bentzen, J. G., Thuesen, L. L., Lauritsen, M. P., Forman, J. L., Loft, A. et al. (2016). Infertile women below the age of 40 have similar anti-Mullerian hormone levels and antral follicle count compared with women of the same age with no history of infertility. *Hum Reprod, 31*(5), 1034-1045.

Ibanez, L., Castell, C., Tresserras, R. and Potau, N. (1999). Increased prevalence of type 2 diabetes mellitus and impaired glucose tolerance in first-degree relatives of girls with a history of precocious pubarche. *Clin Endocrinol (Oxf), 51*(4), 395-401.

Ibanez, L., Ong, K., de Zegher, F., Marcos, M. V., del Rio, L. and Dunger, D. B. (2003). Fat distribution in non-obese girls with and without precocious pubarche: central adiposity related to insulinaemia and androgenaemia from prepuberty to postmenarche. *Clin Endocrinol (Oxf), 58*(3), 372-379.

Ibanez, L., Valls, C., Ferrer, A., Ong, K., Dunger, D. B. and De Zegher, F. (2002). Additive effects of insulin-sensitizing and anti-androgen treatment in young, nonobese women with hyperinsulinism, hyperandrogenism, dyslipidemia, and anovulation. *J Clin Endocrinol Metab, 87*(6), 2870-2874.

Imperato-McGinley, J., Gautier, T., Cai, L. Q., Yee, B., Epstein, J. and Pochi, P. (1993). The androgen control of sebum production. Studies of subjects with dihydrotestosterone deficiency and complete androgen insensitivity. *J Clin Endocrinol Metab, 76*(2), 524-528.

Irez, T., Ocal, P., Guralp, O., Cetin, M., Aydogan, B. and Sahmay, S. (2011). Different serum anti-Mullerian hormone concentrations are associated with oocyte quality, embryo development parameters and IVF-ICSI outcomes. *Arch Gynecol Obstet, 284*(5), 1295-1301.

Ittiwut, C., Pratuangdejkul, J., Supornsilchai, V., Muensri, S., Hiranras, Y., Sahakitrungruang, T. et al. (2017). Novel mutations of the SRD5A2 and AR genes in Thai patients with 46, XY disorders of sex development. *J Pediatr Endocrinol Metab, 30*(1), 19-26.

Khondker, L. and Khan, S. I. (2014). Acne vulgaris related to androgens - a review. *Mymensingh Med J, 23*(1), 181-185.

Kierszenbaum, A. L. and Tres, L. L. (2016). Follicle development and menstrual cycle. In: Kierszenbaum, A. L. and Tres, L. L. (eds.) *Histology and cell biology: an introduction to pathology.* 4th ed. Philadelphia: Elsevier saunders, 665-688.

Kissebah, A. H., Vydelingum, N., Murray, R., Evans, D. J., Hartz, A. J., Kalkhoff, R. K. and Adams, P. W. (1982). Relation of body fat distribution to metabolic complications of obesity. *J Clin Endocrinol Metab, 54*(2), 254-260.

Kuhn, B. H. (1972). Male pattern alopecia and/or androgenic hirsutism in females. 3. Definition and etiology. *J Am Med Womens Assoc, 27*(7), 357-364.

Labrie, F. (2010). DHEA, important source of sex steroids in men and even more in women. *Prog Brain Res, 182*, 97-148.

Lamont, K. R. and Tindall, D. J. (2010). Androgen regulation of gene expression. *Adv Cancer Res, 107*, 137-162.

Leão, R. B. F. and Esteves, S. C. (2014). Gonadotropin therapy in assisted reproduction: an evolutionary perspective from biologics to biotech. *Clinics*, 69(4), 279–293.

Lebbe, M. and Woodruff, T. K. (2013). Involvement of androgens in ovarian health and disease. *Mol Hum Reprod, 19*(12), 828-837.

Lee, K. S., Joo, B. S., Na, Y. J., Yoon, M. S., Choi, O. H. and Kim, W. W. (2000). Relationships Between Concentrations of Tumor Necrosis Factor-α and Nitric Oxide in Follicular Fluid and Oocyte Quality. *J Assist Reprod Genet, 17*(4), 222-228.

Li, R., Cui J., Shen, Y. (2014). Brain sex matters: estrogen in cognition and Alzheimer's disease. *Mol Cell Endocrinol*, *389*, 13–21.

Mason, H. and Franks, S. (1997). Local control of ovarian steroidogenesis. *Baillieres Clin Obstet Gynaecol, 11*(2), 261-279.

McCartney, C. R., Prendergast, K. A., Chhabra, S., Eagleson, C. A., Yoo, R., Chang, R. J. et al. (2006). The association of obesity and hyperandrogenemia during the pubertal transition in girls: obesity as a potential factor in the genesis of postpubertal hyperandrogenism. *J Clin Endocrinol Metab, 91*(5), 1714-1722.

Moghetti, P., Tosi, F., Castello, R., Magnani, C. M., Negri, C., Brun, E. et al. (1996). The insulin resistance in women with hyperandrogenism is partially reversed by antiandrogen treatment: evidence that androgens impair insulin action in women. *J Clin Endocrinol Metab, 81*(3), 952-960.

Nardo, L. G., Yates, A. P., Roberts, S. A., Pemberton, P., and Laing, I. (2009). The relationships between AMH, androgens, insulin resistance and basal ovarian follicular status in non-obese subfertile women with and without polycystic ovary syndrome. *Hum Reprod, 24*(11), 2917-2923.

Nicholas, L. M., Morrison, J. L., Rattanatray, L., Zhang, S., Ozanne, S. E. and McMillen, I. C. (2016). The early origins of obesity and insulin resistance: timing, programming and mechanisms. *Int J Obes (Lond), 40*(2), 229-238.

Norman, R. J. (2002). Hyperandrogenaemia and the ovary. *Mol Cell Endocrinol, 191*(1), 113-119.

Okon, M. A., Laird, S. M., Tuckerman, E. M. and Li, T. C. (1998). Serum androgen levels in women who have recurrent miscarriages and their correlation with markers of endometrial function. *Fertil Steril, 69*(4), 682-690.

Orme, S., Cullen, D. R., & Messenger, A. G. (1999). Diffuse female hair loss: are androgens necessary? *Br J Dermatol, 141*(3), 521-523.

Pache, T. D., Chadha, S., Gooren, L. J., Hop, W. C., Jaarsma, K. W., Dommerholt, H. B. and Fauser, B. C. (1991). Ovarian morphology in long-term androgen-treated female to male transsexuals. A human model for the study of polycystic ovarian syndrome? *Histopathology, 19*(5), 445-452.

Paik, J. H., Yoon, J. B., Sim, W. Y., Kim, B. S. and Kim, N. I. (2001). The prevalence and types of androgenetic alopecia in Korean men and women. *Br J Dermatol, 145*(1), 95-99.

Panidis, D., Tziomalos, K., Papadakis, E., Chatzis, P., Kandaraki, E. A., Tsourdi, E. A. Et al. (2015). Associations of menstrual cycle irregularities with age, obesity and phenotype in patients with polycystic ovary syndrome. *Hormones (Athens), 14*(3), 431-437.

Pinola, P., Lashen, H., Bloigu, A., Puukka, K., Ulmanen, M., Ruokonen, A. et al. (2012). Menstrual disorders in adolescence: a marker for hyperandrogenaemia and increased metabolic risks in later life? Finnish general population-based birth cohort study. *Hum Reprod, 27*(11), 3279-3286.

Pochi, P. E., Shalita, A. R., Strauss, J. S., Webster, S. B., Cunliffe, W. J., Katz, H. I. et al. (1991). Report of the Consensus Conference on Acne Classification. Washington, D.C., March 24 and 25, 1990. *J Am Acad Dermatol, 24*(3), 495-500.

Poretsky, L. (1991). On the paradox of insulin-induced hyperandrogenism in insulin-resistant states. *Endocr Rev, 12*(1), 3-13.

Poretsky, L. and Kalin, M. F. (1987). The gonadotropic function of insulin. *Endocr Rev, 8*(2), 132-141.

Prizant, H., Gleicher, N., and Sen, A. (2014). Androgen actions in the ovary: balance is key. *J Endocrinol, 222*(3), R141-151.

Randall, V. A. (1994). Androgens and human hair growth. *Clin Endocrinol (Oxf), 40*(4), 439-457.

Rebora, A. (2004). Pathogenesis of androgenetic alopecia. *J Am Acad Dermatol, 50*(5), 777-779.

Rice, S., Ojha, K., Whitehead, S., and Mason, H. (2007). Stage-specific expression of androgen receptor, follicle-stimulating hormone receptor, and anti-Mullerian hormone type II receptor in single, isolated, human preantral follicles: relevance to polycystic ovaries. *J Clin Endocrinol Metab, 92*(3), 1034-1040.

Roby, K. F. and Terranova, P. F. (1988). Tumor necrosis factor alpha alters follicular steroidogenesis in vitro. *Endocrinology, 123*(6), 2952-2954.

Rojas, J., Chavez-Castillo, M., Olivar, L. C., Calvo, M., Mejias, J., Rojas, M. et al. (2015). Physiologic Course of Female Reproductive Function: A Molecular Look into the Prologue of Life. *J Pregnancy, 2015*, 715735.

Sá, R. and Sousa, M. (2014) Androgens and the ovary. In: Socorro, S. (ed.) *Androgen Receptors. Structural Biology, Genetics and Molecular Defects.* New York: Nova biomedical; 163-188.

Samara-Boustani, D., Colmenares, A., Elie, C., Dabbas, M., Beltrand, J., Caron, V. et al. (2012). High prevalence of hirsutism and menstrual disorders in obese adolescent girls and adolescent girls with type 1 diabetes mellitus despite different hormonal profiles. *Eur J Endocrinol, 166*(2), 307-316.

Sanchon, R., Gambineri, A., Alpanes, M., Martinez-Garcia, M. A., Pasquali, R. and Escobar-Morreale, H. F. (2012). Prevalence of functional disorders of androgen excess in unselected premenopausal women: a study in blood donors. *Hum Reprod, 27*(4), 1209-1216.

Sen, A., & Hammes, S. R. (2010). Granulosa cell-specific androgen receptors are critical regulators of ovarian development and function. *Mol Endocrinol, 24*(7), 1393-1403. doi:10.1210/me.2010-0006

Shaw, J. C. (2002). Acne: effect of hormones on pathogenesis and management. *Am J Clin Dermatol, 3*(8), 571-578.

Shi, Y., Yang, F., Sun, Z., Zhang, W., Gu, J. and Guan, X. (2017). Differential microRNA expression is associated with androgen receptor expression in breast cancer. *Mol Med Rep, 15*(1), 29-36.

Sonino, N., Fava, G. A., Mani, E., Belluardo, P. and Boscaro, M. (1993). Quality of life of hirsute women. *Postgrad Med J, 69*(809), 186-189.

Taylor, S. I., Dons, R. F., Hernandez, E., Roth, J. and Gorden, P. (1982). Insulin resistance associated with androgen excess in women with autoantibodies to the insulin receptor. *Ann Intern Med, 97*(6), 851-855.

Thomas, D. R. (2004). Psychosocial effects of acne. *J Cutan Med Surg, 8 Suppl 4*, 3-5.

Van Voorhis, B. J., Dunn, M. S., Snyder, G. D., and Weiner, C. P. (1994). Nitric oxide: an autocrine regulator of human granulosa-luteal cell steroidogenesis. *Endocrinology, 135*(5), 1799-1806.

Walker, W. H. (2009). Molecular mechanisms of testosterone action in spermatogenesis. *Steroids, 74*(7), 602-607.

Wang, L. J., Brannstrom, M., Robertson, S. A., and Norman, R. J. (1992). Tumor necrosis factor alpha in the human ovary: presence in follicular fluid and effects on cell proliferation and prostaglandin production. *Fertil Steril, 58*(5), 934-940.

Wei, S., Jones, G., Thomson, R., Otahal, P., Dwyer, T. and Venn, A. (2010). Menstrual irregularity and bone mass in premenopausal women: cross-sectional associations with testosterone and SHBG. *BMC Musculoskelet Disord, 11*, 288.

Wei, S., Schmidt, M. D., Dwyer, T., Norman, R. J. and Venn, A. J. (2009). Obesity and menstrual irregularity: associations with SHBG, testosterone, and insulin. *Obesity (Silver Spring), 17*(5), 1070-1076.

West, S., Lashen, H., Bloigu, A., Franks, S., Puukka, K., Ruokonen, A. et al. (2014). Irregular menstruation and hyperandrogenaemia in adolescence are associated with polycystic ovary syndrome and infertility in later life: Northern Finland Birth Cohort 1986 study. *Hum Reprod, 29*(10), 2339-2351.

Yasmin, E., Balen, A. H. and Barth, J. H. (2013). The association of body mass index and biochemical hyperandrogenaemia in women with and without polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol, 166*(2), 173-177.

Yen, S. S. (1980). The polycystic ovary syndrome. *Clin Endocrinol (Oxf), 12*(2), 177-207.

Zhou, Z., Ni, R., Hong, Y., Li, Y., Wang, Y., Zhao, X. and Yang, D. (2012). Defining hyperandrogenaemia according to the free androgen index in Chinese women: a cross-sectional study. *Clin Endocrinol (Oxf), 77*(3), 446-452.